# First Generation of Controlled-Release Bacteriocins/Anti-Microbials



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Description:

TECHNOLOGY AREAS: Biomedical, Human Systems

ACQUISITION PROGRAM: Combat Feeding Research and Engineering Program

OBJECTIVE: To minimize the threat of bioterrorism and the proliferation of foodborne illness that will adversely affect the performance of the Warfighter by the development of a controlled release mechanism of bacteriocins/anti-microbials to effectively inhibit a broad range of spoilage bacteria, pathogens and spores over the extended shelf life of ration components.

DESCRIPTION: Along with the Warfighter need for a wider variety of higher quality IM ration components there is the ever threatening possibility of bioterrorism and the proliferation of foodborne pathogens (E.coli 0157H: 7, Salmonella spp, and Listeria monocytogenes). This was evident in the US Military “Do Not Consume Recall” (July 2009) of the dairy shake due to the unintended presence of Salmonella. This recall had a far-reaching impact on military subsistence, since the dairy shake is a regular component of Meals Ready-to-Eat, Unitized Group Rations, and Tailored Operational Training Meals that dated back to menus of 2002 and could have resulted in a deadly lesson learned. Bacteriocins/Anti-Microbials added to IM rations in the form of mixed time-release preparations will serve as a biopreservative with the ability to inhibit a wide range of microbes.

Ready to eat products are the mainstay of the military ration platform because Warfighters need high quality components that require little preparation, no refrigeration, and are easily consumable while on the move. Intermediate Moisture (IM) ration components (i.e. Shelf-Stable Sandwiches) are the centerpiece of the First Strike Ration®, however, a wider variety of components are required to keep up with Warfighter demand for variety and prevent menu monotony. Current IM components become noticeably dry or acidic during extended storage. Existing microbial hurdle guidelines restrict a product's pH and water activity (aw) to ensure the safety of IM foods. However, these restrictions do not take into account the impact on a product's organoleptic attributes (i.e., flavor, texture, color, etc) over time. To improve organoleptic attributes, pH and aw will have to be elevated, which in turn makes the IM product susceptible to spoilage and foodborne illness. Current IM components have a pH less than 5.2 with aw below 0.88. The goal of this SBIR is to achieve optimum organoleptics (pH > 5.0, aw > 0.90) by developing a time-released complex of bacteriocins/anti-microbials to give the ration developer greater flexibility with IM product formulations. This will lead to greater ration acceptance, consumption and increased component variety. Further, the innovative development of Controlled-Release Bacteriocins/Anti-microbials will provide a major technological advancement that will assure the safety of IM ration components. All Bacteriocins/Anti-microbials investigated in this study must have a status of Generally Recognized as Safe (GRAS) by the FDA.

The innovative challenge in this SBIR lies in the intricacy of multicomponent food systems, the physical/chemical properties of food materials, and the intermolecular interactions of these bacteriocins/anti-microbials components (i.e., nisin) in intermediate moisture foods. Nano-encapsulation may be an ideal mode of delivery for a complex of bacteriocins; a key ingredient in the first generation of novel biopreserved foods. Nano-technology is one means of providing a controlled release of bacteriocins/anti-microbials, which is vital in keeping levels of the bioactive compounds at an effective concentration over the ration's three year shelf life. This would eliminate the less effective and costly method of over loading the ration with the bioactive up front to account for its likely decrease in effectiveness over the course of the three year shelf life. Conversely, a bacteriocin or complex of bacteriocins or other anti-microbial compounds released in a controlled manner throughout the ration's shelf life will maintain hurdles needed to sustain pathogen inhibition as well as enhance overall acceptability. The utilization of bacteriocins/anti-microbials in hurdle technology may reduce the need of chemical preservatives while also providing a safe high-quality IM ration component resistant to spoilage, pathogens and endospores for its required shelf life under extreme environmental conditions.

Nanoparticle concern has been addressed in many studies where they have been shown to have very limited GI absorption, thus demonstrating low systemic exposure following oral conception (Kreyling et al., 2002). However, the fact remains that by changing nanoparticle properties, such as surface characteristics, the biocompatibility of the particle can be dramatically altered (Stern and McNeil, 2008). According to Gilor et al., 2008, the human GI tract is a balance complex system between the individual and the intestinal microflora that is dominated by two main genera of lactic acid bacteria of which most have means of producing bacteriocins. These same species of lactic acid bacteria are used in the production of most bacteriocins and antimicrobials that are being looked at as future antibiotics and probiotics. Nisin (GRAS) the most commercially used bacteriocin is produced in this manner and studies have shown that it does not possess any sub-chronic or chronic toxicity, reproductive/devolomental toxicity, genotoxicity, or carcinogenicity (Reddy et al., 2004; Hagiwara et al., 2010). This supports earlier findings of Bernbom et al., 2006 where it was reported that the intestinal microbiota in human flora-associated rats was not affected after dosages of nisin in that ingested nisin is easily inactivated by trypsin and pancreatin of which should remain true on the nano scale.

PHASE I: Combat Feeding Directorate is currently exploring new biopreservatives alone and in combination to support this goal, but identifies the need of a controlled release mechanism to maintain the needed activity level throughout shelf-life requirement. Thus, in support of this project, innovative research is needed to explore, develop and design a controlled release mechanism for a complex of bacteriocins. This complex should work in synergy over time to effectively control and kill both gram negative and positive foodborne pathogens, with a release mechanism such as but not limited to nano or micro encapsulation. Thus, phase I should identify potential bacteriocins/anti-microbials, their potential complex mixture, and encapsulation technique for controlled release of the bacteriocins. The control mechanism must release the bacteriocins in a manner that ensures a minimum two year shelf life for military IM ration components. This effort should provide technical specifications for a broad range of nano-encapsulated bacteriocins that are time released but effective against all foodborne pathogens and can be later identified as a commercial-off-the-shelf (COTS) product to be used to develop the first generation of novel biopreserved foods for military feeding.

Identify optimal bacteriocin form to incorporare in an intermediate or low moisture food system. Current forms available include liquid, powder, solid (pellet) or volatile. Potential carriers should be identified to determine bacteriocin delivery in system e.g. sachet, packaging, ingredient. At this time favorable release mechanism (pH, moisture temperature or light) should also be identified. Deliverables will be a final report addressing feasibility and practicality of the proposed concept; technical hurdles that must be achieved as they relate to form, carrier, or release mechanism; associated risks and factors limiting development of a functional prototype of the First Generation of Controlled Released Bacteriocins. Minor exploratory testing may be used to confirm efficacy.

PHASE II: Develop, test and demonstrate a functional prototype of the First Generation of Controlled Released Bacteriocins/Anti-microbials (FGCRB/A) as identified in Phase I. Initially this can be verified by cocktail inoculation studies in broth and media systems. Conduct preliminary test, including accelerated storage studies using model systems or computer simulation, to assess the design and performance of the time release matrix of bacteriocins. Identify potential intermediate moisture ration components that will benefit from this bioactive system and determine optimal way of integrating this additive (micro pellet, powder, liquid) into select matrix or food system. Demonstrate and validate concept by using in an existing ration component, such as a beef/pork wrap, with elevated pH and aw. Demonstrate the efficiency of the FGCRB/A over a range of aw (ie. 0.88, 0.90, 0.95) and pH (ie. 5.0, 5.5, 6.0). Demonstrate the FGCRB/A ability to inhibit pathogen growth (inoculated pack study) under storage conditions of 80F, 100F and 120F. Efficacy should be shown at microbial populations of 102, 104 and 106 colony forming units/ml or gram. Maturity of this food additive technology will ensure the ability to biopreserve a safe, high quality ration component through life cycle and environmental testing to confirm the conceptual design from Phase I. The potential for adverse events will also be documented. This effort will support the development of future ration components. Deliver a report documenting the research effort along with a detailed description of the proposed technique/system and protocol to include specifications and performance of key components.

PHASE III: Produce and deliver prototypes to support technical and user testing in the field with military personnel. Make modifications to most successful prototype based on organoleptic testing and feedback. A small-scale production capability will be established to demonstrate the manufacturing feasibility of the proposed FGCRB/A. Deliver a report documenting the theory, design component specifications, performance characterization and scale-up projection for establishing a large-scale production capability, to include all relevant microbiological data ensuring wholesomeness, safety and adverse event issue if any. The concept meeting the requirement outlined in this effort would be applicable to military feeding, the commercial food industry, space feeding and emergency relief (camping, trucking, disaster relief). A commercialization strategy shall be outlined and a commercialization partner, if required, shall be defined to demonstrate a well-defined path toward commercialization of the FGCRB/A.